REMARKS

Upon entry of the amendment, claims 201, 209, 212-214, 238, 239 and 244 are pending in the application. Claims 202-208, 215-219, 224-237 and 240-243 are cancelled without prejudice. Applicants reserve the right to pursue the subject matter of these claims in one or more continuing applications. Claims 201 is amended and claim 244 is added. Amendments to claim 201 are clerical in nature. Support for claim 244 is found throughout the instant specification, for example in Example 1. No new matter is added.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 201, 209-214, 238 and 239 under 35 U.S.C. 112, first paragraph as lacking proper enablement in the instant specification. The Examiner, while acknowledging that the specification is enabling for:

A method of expanding an *ex-vivo* population of CD34+ and CD34+/CD38- and/or CD133+ hematopoietic stem cells in culture while at the same time inhibiting differentiation of said cells *ex-vivo* in a culture medium, the method comprising:

(a) providing hematopoietic mononuclear cells that are not enriched prior to culturing, culturing said mononuclear cells *ex-vivo* in culture under conditions allowing for proliferation and at the same time inhibiting differentiation, said conditions comprising providing either (i) early acting cytokines selected from the group consisting of SCF, FLT3 ligand, IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, TNF-α and thrombopoietin ; and/or (ii) a late acting cytokine selected from the group consisting of GCSF, G/MCSF, and EPO; and

(b) culturing said mononuclear cells in the presence of the copper chelator TEPA;

thereby expanding the population of said hematopoietic stem cells while inhibiting the differentiation of said hematopoietic stem cells *ex-vivo* in culture;

has alleged that the specification does not provide enablement for culturing mononuclear cells in the presence of any other conditions for proliferation or in the presence of any other copper chelator. Applicants disagree.

Claim 201, from which the remaining claims subject to the rejection depend, is amended herein to delete "at least one copper chelator capable of reducing intracellular available copper concentration" and to recite "at least one copper chelator which reduces

intracellular available copper concentration..." Applicants traverse the rejection with respect to the claims as amended herein.

The Examiner has alleged that the neither the art of record nor the instant specification provide any guidance as to how to practice the claimed method in the presence of any other condition for proliferation or in the presence of any other copper chelator (emphasis added).

Applicants submit that the claims are not directed to any condition for proliferation in the presence of any copper chelator as the Examiner suggests. Rather the claims are directed to culturing mononuclear cells under conditions allowing for cell proliferation by providing these cells with (i) nutrients, (ii) at least an early acting cytokine or cytokines and (iii) at least one copper chelator which reduces intracellular copper concentration in these cells. The exemplary combinations of specific cytokines and the specific copper chelator TEPA recited by the Examiner are merely preferred methods of practicing the instant invention. One of ordinary skill in the art reading the instant specification at the time of filing would be readily capable of making and using the full scope of the invention as claimed without undue experimentation, as described in further detail herein.

The Examiner further asserts that the state of the art at the time of filing shows that the effect of copper chelators on the proliferation of hematopoietic stem cells is unpredictable. As such, the Examiner asserts that one of ordinary skill in the art, wishing to practice the full scope of the invention as claimed, in view of the instant disclosure and the unpredictability in the art, would be required to carry out extensive experimentation to make and use the invention. *See*, Office Action at pages 7-8. Applicants disagree.

First, Applicants submit that the state of the art with respect to the effect of copper chelators on the proliferation of hematopoietic stem cells is not unpredictable as the Examiner suggests. More specifically, Applicants submit that the Examiner has misconstrued the teachings of Percival et al., *Am J Clin Nutr* 67:1064S-1068S, 1998 ("Percival"), on which the Examiner bases the assertion of unpredictability.

The Examiner, citing Percival, states that there is a high level of unpredictability regarding the culture of stem cells and the effects of copper chelation in the art, and that Percival teaches that more work may be required to study the exact role of genus of copper chelator in expansion of HSC. However, Applicants submit that one or ordinary skill in the

art examining pg. 1066S, col. 2, para. 2, cited by the Examiner, in view of Percival as a whole, would readily recognize that Percival is silent regarding any role of any copper chelator in expansion of HSC:

> The opposing question was asked: If copper is removed from the cell, is differentiation impaired or prevented? We hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating. HL-60 cells were incubated with this chelator for 4–21 d before adding the retinoic acid. The amount of copper was reduced by 40–70% and the activity of Cu/Zn SOD was reduced by 60–80% (27). Differentiation was again assessed as the production of superoxide anion. Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, indicating that differentiation had occurred (27). These results were extended by Sergeant and Johnson (28) in HL-60 cells grown in media that had much lower copper concentrations than did ours. In their study, the HL-60 cells were cultured in serum-free medium that contained < 5 nmol Cu/L. The cells differentiated to the same degree as cells grown in fetal bovine serum– or copper-supplemented serum-free medium. Sergeant and Johnson interpreted their results to mean that either the remaining copper in the cells was sufficient for differentiation or the cells were already sufficiently differentiated such that removal of copper did not affect subsequent differentiation. So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, it does not prevent the HL-60 cells from differentiating.

Rather, Percival questioned whether their "TEPA model" of copper chelation is useful in studying the effect of copper on induced differentiation in the CD34-/CD133- HL-60 cell line, concluding that their "TEPA model" is not useful. No studies of expansion of CD34+ or CD133+ HSC are taught or suggested by Percival. Thus, Applicants submit that the skilled artisan would readily recognize that Percival does not provide any support to the Examiner's assertion that the use of various copper chelators in the claimed methods is unpredictable.

Second, Applicants submit that one or ordinary skill in the art would not require undue experimentation to practice the claimed invention as the Examiner asserts. As stated by the Examiner, the instant specification teaches culturing mononuclear cells under conditions allowing for cell proliferation by providing these cells various nutrients and

cytokines and by providing at least one copper chelator which reduces intracellular copper concentration in these cells (*e.g.*, TEPA). The instant inventors have repeatedly shown that copper chelators which reduce intracellular available copper concentration can effectively inhibit differentiation of stem and progenitor cells in *ex-vivo* culture. Good correlation has been found between the ability of chelators to modulate cellular copper content and their biological activities: chelators that reduce cellular copper content are potent differentiation inhibitors. Differentiation inhibitory chelators, such as TEPA, PEHA etc., were found to inhibit differentiation. *See*, for example, U.S. Patent No. 6,962,698 at Table 3.

It will be further appreciated that the instant inventors have demonstrated that the addition of a transition metal chelator having affinity for copper and capable of reducing available intracellular copper effectively inhibits differentiation of stem and progenitor cells cultured in a variety of combinations of cytokines and nutrients. *See*, for example, U.S. Patent No. 6,962,698 at Example 1, Table 1, Figures 12-14 and Figure 20.

Moreover, methods of culturing cells with copper chelators were well known in the art at the time of filing the instant application, as were devices and methods for assessing the effect of copper chelators on available intracellular copper of cells (*e.g.*, hematopoietic cells). *See*, for example, Bae and Percival, *J. Nutrition*, 123:997-1002, 1993; Weston et al, *J. Immunol Methods* 133:87-97, 1990 and Weston at al, *Cytometry* 13:739-49, 1992.

It is well understood under U.S. law that the quantity of experimentation needed to be performed by one skilled in the art is only one factor involved in determining whether "undue experimentation" is required to make and use the invention. An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance. *In re Colianni*, 561 F.2d 220, 224 (CCPA 1977). The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 858 F.2d. 731, 737 (Fed. Cir. 1988). Time and difficulty of experiments are not determinative if they are merely routine. *Id.*

The methods of Bae and Percival and Weston et al. are readily adaptable to high-throughput screening, and thus large numbers of copper chelators can be routinely assessed, quickly and effectively, by the skilled artisan. Thus, one of ordinary skill in the art could

readily determine the actual effect of any nutrient, any early acting cytokine and any copper chelator on the available intracellular copper of cells, as required by the instant claims, without engaging in undue experimentation.

Lastly, the Examiner assets that the breath of the instant invention contemplates the use of certain copper chelators, such as EDTA or citrate, which may also chelate iron and cationic minerals necessary for cell proliferation. *See*, Office Action at page 7. Applicants submit that although the instant specification describes copper chelate or chelators as indicated by the examiner at page 54 and page 66 of the instant specification, the instant claims require that the mononuclear cells are cultured *ex-vivo* "under conditions allowing for cell proliferation". Conditions for cell proliferation are easily assessed by one of ordinary skill in the art. *See*, for example, the instant specification at Example 1, Experimental Procedures and U.S. Patent No. 7,169,605. Thus, chelators incompatible with cellular proliferation are readily identified by the skilled artisan and are not encompassed by the claimed invention.

Based on the foregoing, Applicants submit that the pending claims, as amended herein, are fully enabled by the instant specification such that one of ordinary skill in the art could make and use the invention without undue experimentation. Reconsideration and withdrawal of the present rejection are respectfully requested.

35 U.S.C. § 112, Second Paragraph, Rejections

Claims 201, 209-214, 238 and 239 are rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Claims 201 (from which the remaining claims subject to the rejection depend) is amended. Applicants traverse the rejection with respect to the claims as amended herein.

The Examiner has rejected claim 201 for reciting "at least one copper chealtor capable of reducing intracellular available copper concentration in said cell" asserting that "capable of reducing intracellular available copper concentration" is a latent property that renders the claim unclear. Claim 201 is amended herein to recite " at least one copper chealtor which reduces intracellular available copper concentration in said cell."

Applicants submit that the pending claims, as amended herein, point out and distinctly claim the subject matter which the Applicant regards as his invention and that one of ordinary skill in the art would readily determine the metes and bounds of the subject matter claimed herein. Reconsideration and withdrawal of the present rejection are respectfully requested.

35 U.S.C. § 103 Rejection

Claims 201, 209-214 and 238-239 are rejected under 35 U.S.C. §103(a) as being unpatentable over Sandstrom et al Blood, 1995;86(3):958-70 ("Sandstrom") and WO 99/40783 to Peled et al. ("Peled"). The Examiner has alleged that Sandstrom teach the *ex-vivo* expansion of CD34+ cells from unselected MNC and CD34+ enriched sources, and that both types of cultures produced similar types of cells after 10 days of culture. The Examiner thus asserts that one of ordinary skill in the art would be motivated by Sandstrom to use the methods of expansion of hematopoietic stem cells disclosed and taught by Peled to expand CD34+ cells from unselected mononuclear cells. Applicants traverse.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. Further, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Applicant submits that the Examiner has failed to meet the burden to establish a *prima facie* case of obviousness of the claimed invention over the combination of Sandstrom and Peled, as required *supra*.

One of ordinary skill in the art, reading Sandstrom as a whole, would readily recognize that no net expansion of CD34+ cells was achieved in either the MNC or CD34+ selected cultures. Under all cell culture conditions taught by Sandstrom, although total cell number in both MNC and CD34+ selected cultures showed a tendency to increase during up to 15 days culture (see Table 2), measurement of the CD34+ fraction of the cultures clearly

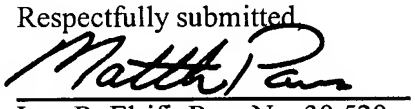
shows marked loss of CD34+ cells in both the MNC and CD34+ selected cultures, regardless of the use of perfusion or static reactors. *See*, for example, Sandstrom, Table 5 and Table 1. Thus, contrary to the Examiner's assertions, Sandstrom does not teach or suggest the expansion of CD34+ cells as required by the instant invention. In stark contrast, one of ordinary skill in the art would readily recognize that the claimed methods of the instant invention clearly achieve net expansion of the CD34+ cell population from both CD34+ selected and unenriched MNC cell cultures in both the short term and long term (up to 12 weeks or more), as evidenced by Figures 1a, 1b, Figure 2 and Table 1 of the instant specification.

Peled does not cure these deficiencies of Sandstrom. Peled does not teach or suggest the expansion of CD34+ cells from an unselected MNC population. As such, Applicants submit that the combination of Sandstrom and Peled fail to teach or suggest all the limitations of the claimed invention, and as such, do not constitute prima facie evidence for obviousness. Further, Applicants submit that one of ordinary skill in the art reading the combination of Sandstrom and Peled would have no reasonable expectation of success in reaching the claimed invention. Reconsideration and withdrawal are respectfully requested.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



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